

Faculty of Science

THE EFFECT OF REDUCED SNOW COVER AND SUMMER DROUGHT ON TEMPERATE GRASSLANDS IN THE SOUTHERN INTERIOR OF BRITISH COLUMBIA

2016 | JANELLE PAULSON

B.Sc. Honours thesis



**THE EFFECT OF REDUCED SNOW COVER AND SUMMER DROUGHT ON
TEMPERATE GRASSLANDS IN THE SOUTHERN INTERIOR OF BRITISH
COLUMBIA**

by

JANELLE PAULSON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF SCIENCE (HONS.)

in the

DEPARTMENT OF BIOLOGICAL SCIENCES
(Ecology and Environmental Biology)



This thesis has been accepted as conforming to the required standards by:

Lauchlan Fraser (Ph.D.), Thesis Supervisor, Dept. Natural Resource Sciences

Christine Petersen (M.Sc.), Co-supervisor, Dept. Biological Sciences

Matt Reudink (Ph.D.), Examining Committee member, Dept. Biological Sciences

Dated this 9th day of May, 2016, in Kamloops, British Columbia, Canada

© Janelle Patricia Paulson, 2016

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Lauchlan Fraser for support and guidance over the course of this project, the International Drought Experiment for coordinating groups around the globe and making this long term project possible, and my Honours committee; Christine Petersen and Dr. Matt Reudink for reviews, suggestions and advice.

Field assistants and lab members in the Fraser Lab helped build experimental plots during the hot summer; Jessica Hewitt, Matthew Coghill, and Sierra Rae. Lastly, a special thanks to Dr. Heath Garris for his suggestions and encouragement throughout every step of this project.

ABSTRACT

Approximately 26% of the Earth's total land area and 80% of agricultural land is composed of grasslands. Lac du Bois Grasslands Protected Area near Kamloops, BC, Canada is temperate grassland that provides recreational and economic opportunities. Due to global climate change, Southern and central British Columbia precipitation patterns are predicted to shift with less precipitation in the summer and more in the winter. Although winters are predicted to become wetter, less precipitation as snowfall could lead to reduced snowpack, which is more likely to melt earlier in the spring. Snow cover provides insulation against cold air, which helps reduce frost stress during the winter and an earlier snowmelt may lead to water shortage in the summer. Changing climate trends may lead to more frequent and intense droughts combined with increased frost stress that can negatively impact plant survival and productivity. However, it is unknown how these stress events interact with each other and if there is a positive or negative relationship. This study looked at the effects of frost stress and drought, as well as the combination of both, on biomass productivity. I found that snow removal increased exposure to more variable and more negative temperatures in the winter. Plots with established rain-out shelters showed a significant decrease in soil moisture content. Aboveground biomass did not differ between plots, treatments types or the controls. Although no significant results were found in biomass production, understanding how stress events interact with each other on grassland plant communities will help predict how terrestrial ecosystems will respond in the face of global climate change.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
ABSTRACT.....	iii
TABLE OF CONTENTS.....	iv
LIST OF FIGURES	v
Introduction.....	1
Climate change on grassland communities	2
Ecological stress memory.....	3
Possible mechanisms of stress imprinting.....	4
Drought tolerance and memory	5
Frost tolerance and memory	6
Cross-stress memory	7
Materials and Methods.....	8
Study site	8
Experimental design.....	9
Rain-out shelter construction	10
Measurements.....	13
Statistical analysis	13
Results.....	14
Snow removal.....	14
Aboveground biomass.....	17
Soil moisture content.....	18
Discussion	20
Snow removal.....	21
Aboveground biomass.....	22
Soil moisture content.....	23
Conclusions	23
LITERATURE CITED	25

LIST OF FIGURES

Figure 1. Lac du Bois Grassland Provincial Park boundary outlined in red. The study site is located in the upper grasslands and is shown by the marked box.	9
Figure 2. Experimental plot design. Six 3 x 3 m plots were established, three control and three rain-out shelters. Within each plot there are treatment and control subplots.....	10
Figure 3. Measurements and final design of constructed rain-out shelters.....	11
Figure 4. Rain-out shelters at the study site in the upper grasslands of Lac du Bois. Gutters move water away from the plots and trenching helps hydrologically isolate plots from each other.....	12
Figure 5. Dates of snow removal from November 2014 to January 2015. Mean snow depth before and after removal.	14
Figure 6. Mean snow depth before and after removal. Asterisk indicates a significant difference in snow depth after removal. Bars are \pm Standard Error.....	15
Figure 7. Temperature comparison between snow removal treatment plots and control plots. Treatment plots have more negative and variable temperature compared to the control.	16
Figure 8. Accumulated frost stress degree days of the treatment and control plots from November 2014 to August 2015.	17
Figure 9. Aboveground biomass collected in November 2015. No difference in biomass was found between the plots and treatment types.	18
Figure 10. Pre rain-out shelter and control soil water content recorded by data loggers from November 2014 to August 2015. This demonstrates that there is little variance in moisture between plots.	19
Figure 11. Soil moisture content determined from soil cores collected in November 2015. Different letters indicate significant differences between treatment types.	20

Introduction

Approximately 26% of the Earth's total land area and 80% of agricultural land is composed of grasslands (Bovel and Dixon 2012). Grasslands are defined as areas dominated by grasses with less than 10% tree cover (Wikeem and Wikeem 2004). Lac du Bois Grasslands Protected Area (GPA) near Kamloops, British Columbia, Canada is temperate grassland with highly palatable forage for cattle and excellent recreational opportunities (Ministry of Environment 2004). Its close proximity to the City of Kamloops has provided locals with easy access for a variety of uses such as research, bird watching, all-terrain vehicle use, mountain biking and hiking (Ministry of Environment 2000). Lac du Bois is important for conservation as it represents the vast biodiversity of the Thompson Basin and Northern Thompson Upland Ecosections (Ministry of Environment 2004). In the park there are lakes, ponds, wetlands, forests and glacial landscape features which provide a diverse environment for species to inhabit (Ministry of Environment 2000). According to the Conservation Data Centre (COSEWIC 2001) about 45 wildlife species, 11 plant species and 13 plant communities in the Lac du Bois ecosystem are Red or Blue-listed. Red-listed species are threatened or endangered and Blue-listed species are vulnerable (Ministry of Environment 2004). Furthermore, Lac du Bois provides habitat for migratory birds and sustains large populations of bighorn sheep and deer (Ministry of Environment 2004). There is an elevational gradient within the park that corresponds with changes in grassland plant communities and increasing moisture (Lee et al. 2014). These communities are known as the lower, middle and upper grassland; each with distinctive soil types and species composition which contributes to high species diversity (van Ryswyk et al. 1996). It is important to protect these species to help maintain biodiversity for a healthy ecosystem that can be enjoyed by visitors for many generations to come.

Grasslands cover about a quarter of the Earth's ice free land surface and have high soil carbon content, which therefore is an important factor in the global carbon balance (Jérôme et al. 2014). Agriculture, forestry and other land use (AFOLU) contribute about 12% of greenhouse gas (GHG) emissions and fossil fuel combustion contributes about 78% of carbon emissions (IPCC 2012). Carbon, from fossil fuels, is released into the atmosphere but forests and grasslands can mitigate 10-40% of emissions every year (Sun et al. 2015). Not only are grasslands important for biodiversity and livestock production, they are also crucial for reducing the carbon imprint of greenhouse gases to help mitigate the effects of climate change (Batalla et al. 2015).

Climate change on grassland communities

From 1906 to 2006, mean global surface air temperature has risen between 0.56 °C and 0.92 °C (IPCC 2007). Temperature changes, due to increased greenhouse gas emissions, are predicted to cause shifts in precipitation patterns, seasonal runoff and evapotranspiration (Nalley et al. 2013). Shifts in precipitation in Southern and Central British Columbia are predicted to have less precipitation in the summer and more in the winter (Spittlehouse 2008). In grasslands, water availability is a major limiting factor for above-ground biomass production (Naudts et al. 2013; Reichmann and Sala 2014). Precipitation is important in Lac du Bois Grasslands Protected Area (GPA) as there is an elevational gradient of plant communities that correspond with increasing elevation and moisture. With the prediction of warmer and drier growing seasons, grassland communities may change drastically and expand northward or into forested areas (Lee et al. 2014). Extreme weather events, such as droughts, are predicted to increase in frequency and magnitude (Yusa et al. 2015). When exposed to drought, some plants respond by decreasing stomatal conductance (Fraser et al. 2009). This reduces water loss by restricting the size, or

altering the density, of the stomata which limits the amount of carbon uptake during photosynthesis (Chaves et al. 2002). With the main source of carbon limited, plants must rely on stored reserves to meet their energy requirements (Chapin et al. 2002). This can increase plant susceptibility to mortality after long periods of time and shift spatial distributions of plant species. Non-native species may take advantage of gaps in plant communities and invade a vacant niche leading to increased competition with native species (Fraser and Carlyle 2011; Holzmüller and José 2013; Kleunen et al. 2015). Many exotic species exist in B.C's grasslands and may increase in abundance with changing climate to further alter community composition and reduce diversity (Lee et al. 2014).

As a result of global climate change there is less precipitation in the form of snowfall (Briceño et al. 2014). Although winters are predicted to become wetter, less precipitation as snowfall leads to reduced snowpack which will melt earlier in the spring (Spittlehouse 2008). Snow cover provides insulation against cold air which helps reduce frost stress to survive during the winter (Briceño et al. 2014). An earlier snowmelt may lead to water shortage and drought during the summer resulting in detrimental effects on biomass production (Choler 2015). Independently, frost and drought may negatively impact plant survival and productivity (Medeiros and Pockman 2011; Smith 2011). However, plants exposed to recurrent stress may be able to adapt better to subsequent stress (Backhaus et al. 2014).

Ecological stress memory

When abiotic and biotic stresses occur, plants must be able to respond and adapt quickly as they are immobile and unable to relocate (Kinoshita and Seki 2014). Plants show some phenotypic plasticity as they acclimate to changing conditions (Fraser et al. 2009; Walter et al. 2011). This plasticity may prime the plant and cause a “stress imprint” that improves defence

response to future stress events (Bruce et al. 2007). Walter et al. (2013) call this phenomenon ecological stress memory. However, it should be noted that the term “memory” does not mean plants are cognisant but are instead stress imprinted (Bruce et al. 2007). Ecological stress memory differs from acclimation as it remains long after the stress has been applied which allows the plant to respond faster to recurrent stress (Walter et al. 2013). However, ecological memory may also involve acclimation mechanisms and the accumulation of protective substances to increase persistence (Kinoshita and Seki 2014; Walter et al. 2013).

Possible mechanisms of stress imprinting

Stress imprints are made through the process of priming by initiating a state of readiness to allow for a faster response to an environmental cue (Bruce et al. 2007; Frost et al. 2008). Conrath et al. (2006) propose two potential mechanisms involved with priming. The first mechanism for priming involves the accumulation of signalling proteins or transcription factors and the second mechanism involves epigenetic changes (Blackhaus et al. 2014; Bruce et al. 2007). Signalling proteins that are accumulated may be activated by post-translational modification of calcium dependent protein kinases when exposed to stress (Conrath et al. 2006; Pastor et al. 2013). For example, a mutant with an affected cyclin-dependent kinase-like protein has reduced salicylate defenses against microbial pathogens and abiotic stressors (Ton et al. 2005). Therefore, the accumulation of cyclin-dependent kinase may promote the salicylate-dependent defence pathway (Bruce et al. 2007). However, signalling proteins are limited by their turn-over times and are not suitable for long-lasting resistance and defence (Pastor et al. 2013). The accumulation of transcription factors may increase defence gene transcription after being exposed to stress (Bruce et al. 2007). An example is shown when *Arabidopsis* is exposed to cold stress. A stress-induced transcription factor gene, known as HOS10, encodes MYB

transcription factors that are crucial to cold acclimation but also affects drought tolerance by controlling abscisic acid (ABA) biosynthesis (Bruce et al. 2007; Yamaguchi-Shinozaki and Shinozaki 2006). Thus, the accumulation of transcription factors help with response time to multiple stressors. Priming effects through epigenetic changes may lead to longer lasting stress imprints (Bruce et al. 2007). These changes involve DNA methylation which can influence seed development, vernalization, and organization of apical meristem all of which can be inherited (Bruce et al. 2007; Kinoshita and Seki 2014). Modifications of chromatin may leave a record of gene activity as epigenetic marks that can act as transgeneration stress memories for faster responses to future attacks (Ćuk et al. 2010).

Drought tolerance and memory

When exposed to drought, plants must be able to quickly acclimate and increase their tolerance. Some mechanisms involve the accumulation of osmoprotective proteins (dehydrins), soluble sugars or the reduction in photosynthetic machinery (Bohnert 2000; Chaves et al. 2002). Morphologically, the root to shoot ratio increases when undergoing drought for access to deeper soil layers for moisture (Sanaullah et al. 2012). ABA-induced genes are important during dehydration to partially close stomata and reduce water loss (Fleta-Soriano and Munné-Bosch 2016). Previous work found that repeated drought improved photoprotection to manage reactive oxygen species compared to plants exposed to a single drought event (Walter et al. 2011). Another study looking at antioxidative enzymes found that plants exposed to drought had decreased enzymatic activity of catalase and ascorbate peroxidase which was passed on to their progeny (Ćuk et al. 2010). This suggests that some plants are able to transmit information about stress exposure to their progeny and that some physiological processes are modified by multi-stress events (Walter et al. 2013).

Frost tolerance and memory

During sub-zero temperatures, ice crystals may form within intracellular spaces or dehydration may occur resulting in damage to the photosynthetic machinery (Roychoudhury et al. 2015; Medeiros and Pockman 2011). Frost acclimation involves similar mechanisms to drought acclimation such as the accumulation of osmoprotective proteins (dehydrins) and soluble sugars (Walter et al. 2013). Transcription of late embryogenesis abundant (LEA) genes may also occur during frost acclimation to help prevent damage from desiccation and cold (Yamaguchi-Shinozaki and Shinozaki 2006). Frost hardening takes several weeks but dehardening can occur within hours if the temperature increases (Walter et al. 2013). This leaves the plant vulnerable to frost damage if the temperature is variable. Although the frequency of frost days is predicted to decrease and mean winter temperature is predicted to increase, extreme cold events will still occur (Kodra et al. 2011). This can be detrimental to plant communities if snow cover depth is too low to provide enough insulation against cold air temperatures. The combination of variable temperature, reduced snow cover and extreme cold events may intensify frost stress over the years (Walter et al. 2013). Being able to acclimate to frost stress and stress imprint for future winters would be advantageous especially with the predicted trends of climate change. A study done by Tahkokorpi et al. (2007) found that after being exposed to frost stress in the winter, newly grown stems of bilberry in the spring showed higher amounts of anthocyanin used for protection against cold temperatures, desiccation and high light. However, a study done by Polle et al. (1996) found that frost tolerance and resistance was lowered after exposure to frost stress. Overall, the response to recurrent exposure of frost stress on tolerance has not been thoroughly investigated and it remains unclear if frost stress experience helps survival (Walter et al. 2013).

Cross-stress memory

Drought and frost have similar acclimation mechanisms to help with water loss and dehydration (Beck et al. 2007). Thus, it may be possible for the formation of stress memory to one kind of stress that also improves the tolerance to different stress resulting in cross-stress memory (Walter et al. 2013). A study by Medeiros and Pockman (2011) found that drought improved freezing tolerance in leaves of *Larrea tridentata*. Another study by Kreyling et al. (2012) found that prior exposure to drought improved late frost tolerance of three out of four grass species tested. There are some studies investigating the interactions of drought on frost tolerance but research of frost on drought tolerance has not been thoroughly investigated. Previous studies have also been limited in time and there are few long term studies looking at ecological stress memory (Bruce et al. 2007; Walter et al. 2013).

The International Drought Experiment has organized a vast network of scientists across the globe to participate in a five year study that investigates the long term effects of drought sensitivity when exposed to frost stress (International Drought Experiment 2015). This experiment may provide more information on the mechanisms involved with ecosystem response to recurrent stressors and if this response has a positive or negative effect on plant communities. I asked the following questions (1) What are the effects of snow removal on biomass productivity and soil temperature? (2) What are the effects of the rain-out shelters and drought on biomass productivity and soil moisture? (3) Will aboveground biomass production improve when exposed to multiple stress events compared to a single event?

Materials and Methods

Study site

This study was conducted in Lac du Bois Grasslands Protected Area, British Columbia, Canada (Figure 1). Lac du Bois is a recreational area comprised of 15,207 ha of valley slopes that includes an elevational gradient of lower, middle and upper grasslands (Ministry of Environment 2004). These vegetation zones change with increasing moisture and primary productivity over its elevational range of 300-975 m above sea level (asl) (Lee et al. 2014). Lac du Bois represents three biogeoclimatic zones (BEC) including the Bunchgrass zone (BG), Ponderosa Pine zone (PP) and Interior Douglas-fir zone (IDF) (Ministry of Environment 2000).

The study site is located in the upper grasslands at 900 m asl (50°47'20.4"N 120°26'53.2"W). Soils are classified as Black Chernozems and rough fescue (*Festuca campestris*) is the dominate species (Ryswyk et al. 1966).



Figure 1. Lac du Bois Grassland Provincial Park boundary outlined in red. The study site is located in the upper grasslands and is shown by the marked box.

Experimental design

Cattle exclosures (30 m x 30 m) were erected by the Agriculture and Agri-Food Canada Kamloops office in April 2010 (McCulloch 2013). HOBO® Micro Station Data Loggers were set up in November 2014 to record soil temperature and soil moisture every fifteen minutes. Snow was removed from the snow removal subplots using a shovel and leaf blower in November to January 2015 to stimulate frost stress. There were six experimental plots containing subplots with two treatments, snow removal and the control. Three of the six plots had a rain-out shelter

and the others did not. These subplots were 1 m x 1 m to allow for a 0.5 m buffer between the sides resulting in a 3 m wide experimental plot (Figure 2). The rain-out shelters were built within the exclosures in May, 2015.

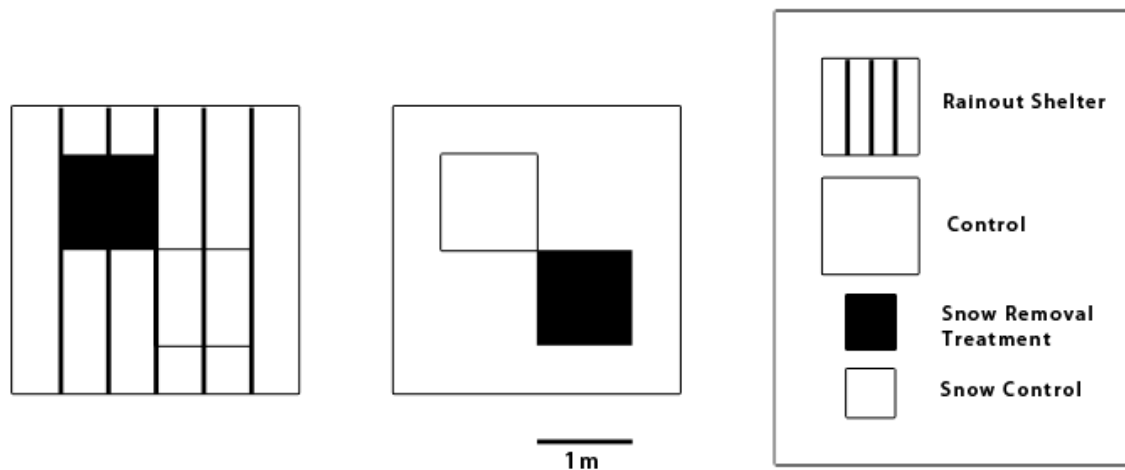


Figure 2. Experimental plot design. Six 3 x 3 m plots were established, three control and three rain-out shelters. Within each plot there are treatment and control subplots.

Rain-out shelter construction

The shelters were constructed to control for the prevailing winds (North) with the slope facing South. Post holes were dug and 4x4x8 feet pieces of commercially treated wood were concreted in each corner of the plot. The supports of the structure consisted of 2x4x10 feet pieces of wood with the side pieces placed at 1.3 m above grade. The upper support of the shelter was placed at 1.6 m above grade and the lower support was placed at 1 m above grade. Two support beams across the plot were screwed on the side supports at 80 cm and 175 cm from the top. These cross beams provided additional support for the SUNTUF® Corrugated Polycarbonate to gently rest on (Figure 3). All supports and posts were levelled and straightened. The

polycarbonate was cut into strips containing two troughs (roughly 14 cm) then screwed onto all of the supports. Glue was used on the screws in the polycarbonate to prevent any leaks. The strips were spaced 14 cm apart to reduce rainfall by 50% to stimulate drought stress. The intensity of rainfall reduction was determined between the 1st and 5th percentile of the precipitation historical record spanning over 50 years in Kamloops. The unmanipulated (control) plots were not covered by rain-out shelters. These structures were built for a long-term study and are expected to last at least five years. Construction of the shelters was completed in late August. Finally, trenching around all of the plots occurred in September to hydrologically isolate them from each other (Figure 4).

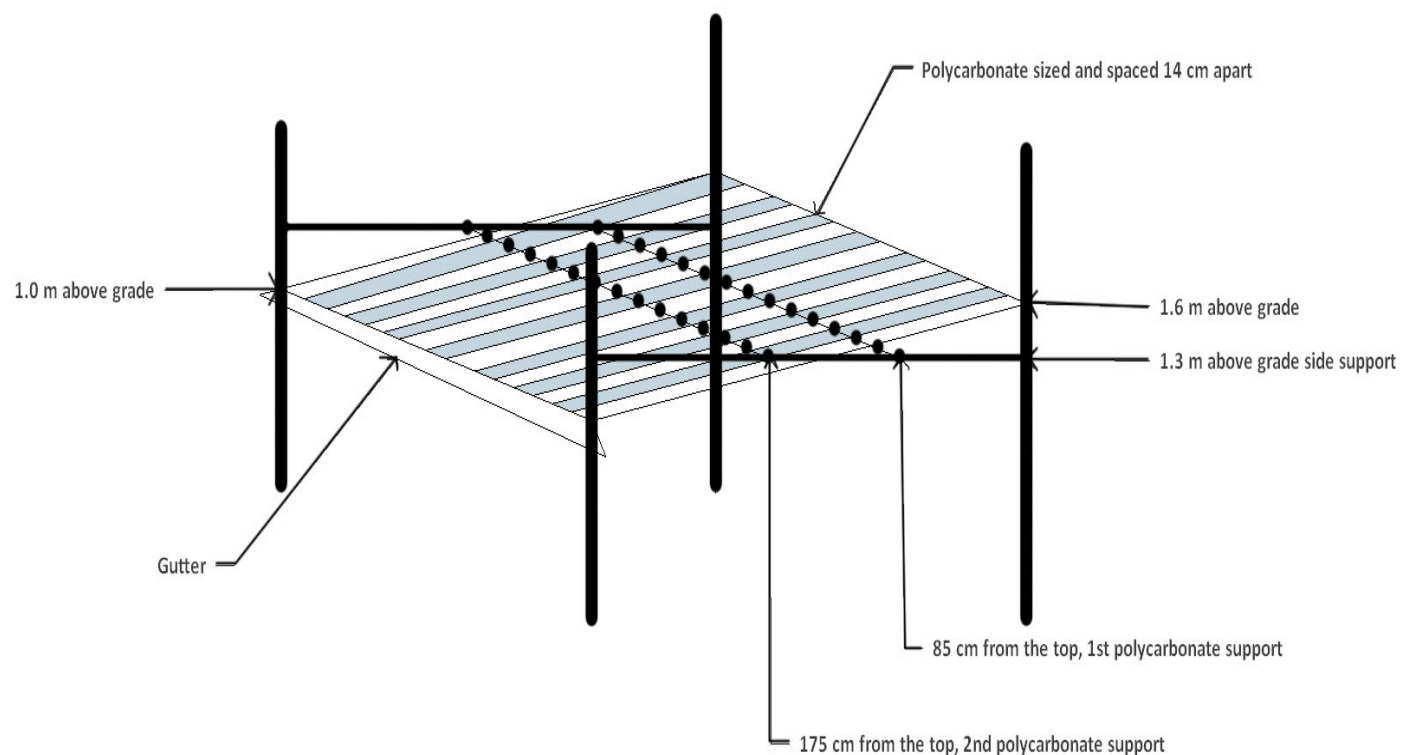


Figure 3. Measurements and final design of constructed rain-out shelters.



Figure 4. Rain-out shelters at the study site in the upper grasslands of Lac du Bois. Gutters move water away from the plots and trenching helps hydrologically isolate plots from each other.

Measurements

A plant survey was conducted in June where the plant species composition was estimated to the nearest 1% using a modified Daubenmire method with 1 x 1 m quadrats placed in the middle of the plot. Percent cover for litter, bare soil, animal diggings and rocks was also estimated. Slope and aspect were determined using a clinometer. Due to the delayed construction of the plots, an aboveground biomass collection was conducted in November as an attempt to allow for fall regrowth and some stress interactions to occur. The biomass and litter was collected in 10 x 100 cm strips in each subplot as advised by the Nutrient Network (2008). The collected biomass was dried at 60°C for two days then weighed. At the same time as biomass collection, soil cores were collected from each plot and dried then weighed to determine soil moisture content. Moisture content and temperature were recorded every fifteen minutes starting from November 2014. The data loggers were placed in a plastic container in the center of each plot to prevent water damage and animal interference.

Statistical analysis

The study design allowed for two-way ANOVAs to test the effect of snow removal (present/absent) and drought (rain-out/control) on biomass and soil moisture. To meet the ANOVA assumption of equality of variances, data were natural log or square root transformed. A one-way ANOVA was done on mean snow depth before and after removal. Accumulated Frost Stress Degree Days (FSDD) was calculated to determine the difference in frost stress contribution between the control and snow removal plots. Average temperature below zero of the snow removal and control plots were analyzed as it represents exposure to frost stress. The daily contribution to frost stress was summed over the course of the year to show the accumulation of frost stress. A paired t-test was done on the control and snow removal FSDD means.

Results

Snow removal

Starting in November and ending in January, snow was repeatedly removed from snow removal subplots within experimental plots (Figure 5). Mean snow depth was square root transformed and analyzed by one-way ANOVA.

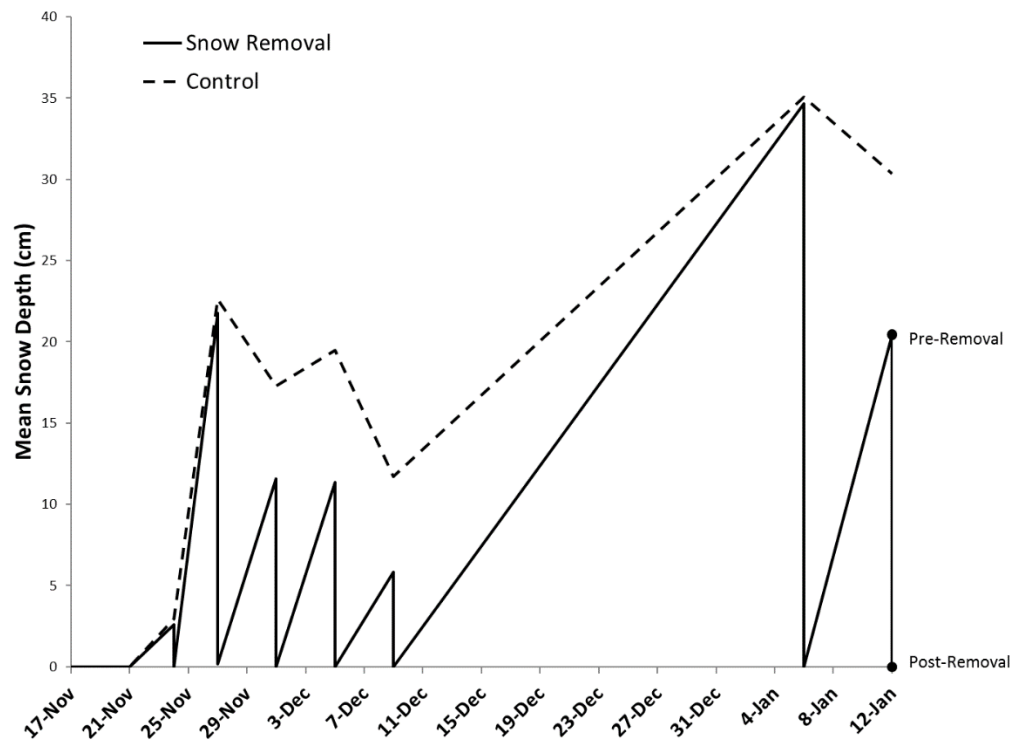


Figure 5. Dates of snow removal from November 2014 to January 2015. Mean snow depth before and after removal.

Removing snow showed a significant reduction in mean snow depth shown in Figure 6 ($F= 19.038$, $df= 3$, $p \leq 0.001$). An extremely heavy snowfall occurred in January creating deep snow cover in the control and treatment plots. It was crucial to remove snow repeatedly

throughout the winter to maintain exposure to frost stress and prevent snow build up in treatment plots.

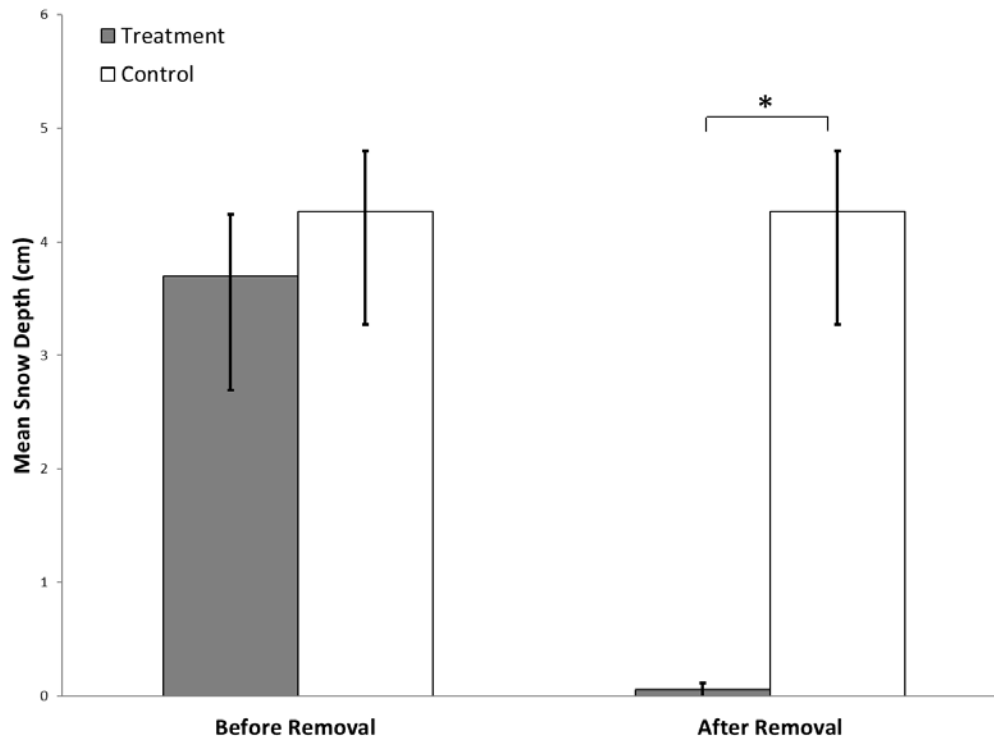


Figure 6. Mean snow depth before and after removal. Asterisk indicates a significant difference in snow depth after removal. Bars are \pm Standard Error.

Snow removal increased exposure to more variable and negative temperatures with a low of -8.1°C (Figure 7). Control plots had a low of -2.9°C and appeared to have less variance in temperature throughout the winter. This demonstrates increased insulation found with higher snow cover and depth.

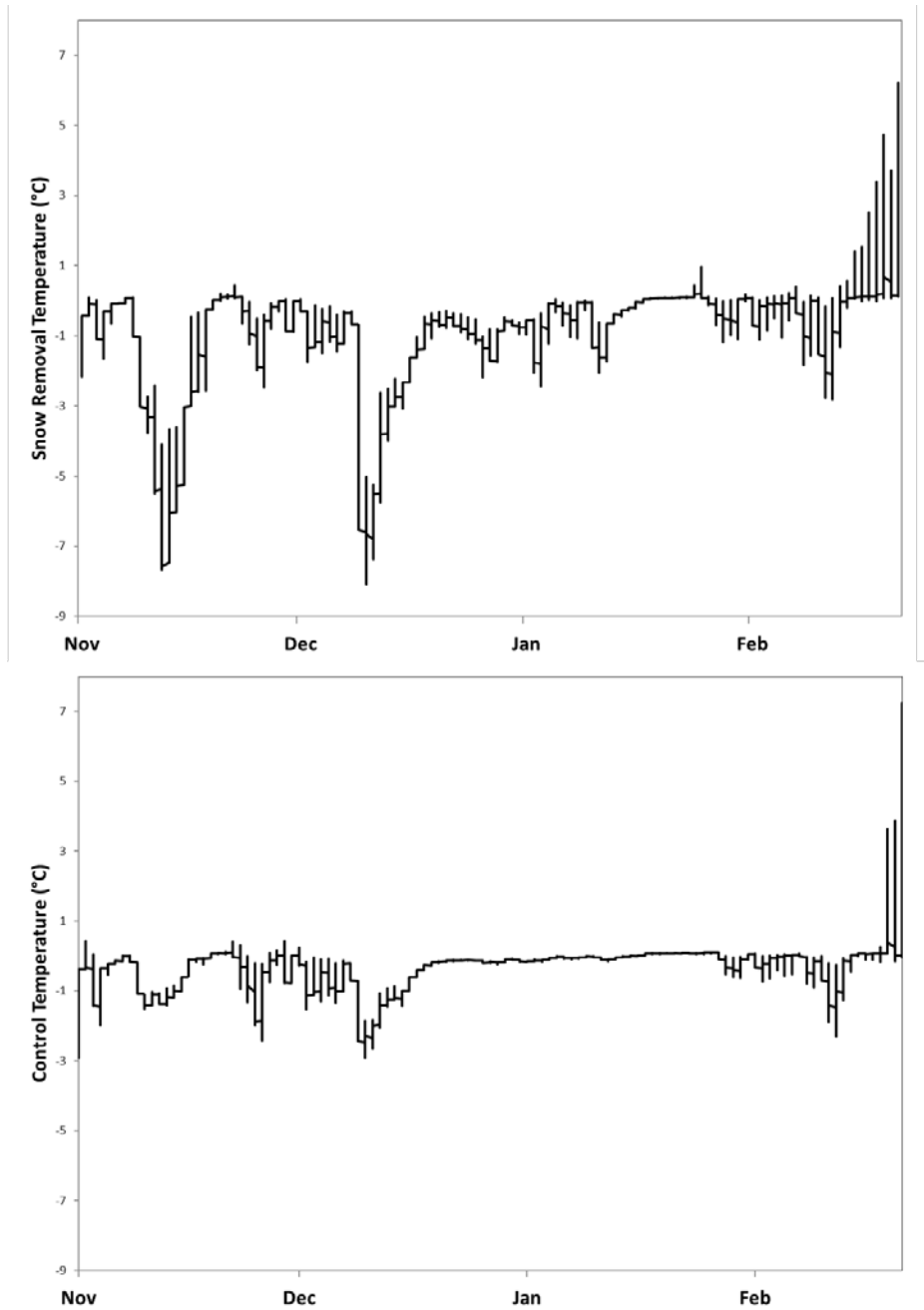


Figure 7. Temperature comparison between snow removal treatment plots and control plots. Treatment plots have more negative and variable temperature compared to the control.

Accumulated FSDD was calculated between November 2014 and August 2015 (Figure 8). There was an error in one probe at a snow removal plot which inaccurately logged temperature after the spring. Calculations were made without the defective plot temperature. The

snow removal plots accumulated FSDD for a total of 105.5 °C compared to the control plots of 34.8 °C. A high FSDD represents increased exposure to negative temperatures and potentially more damage. Snow cover insulated control plots from sub-zero temperatures reducing the accumulation of frost stress. A paired-t test was done on the mean FSDD of snow removal and control plots where a significant difference was found ($t_4 = 2.77$, $p = 0.022$).

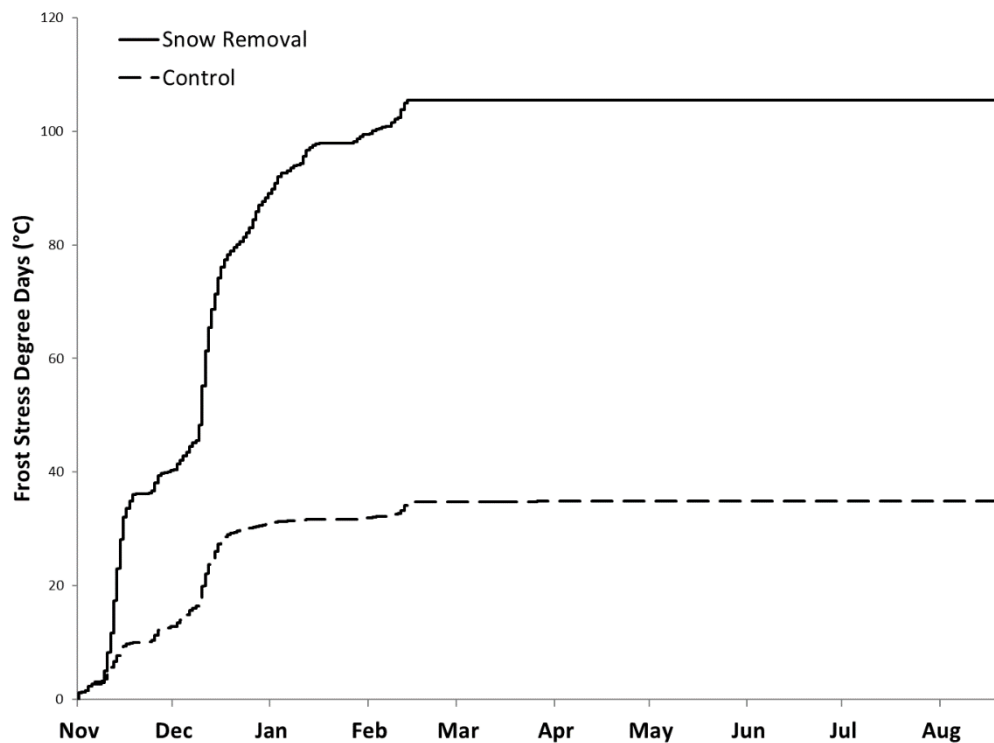


Figure 8. Accumulated frost stress degree days of the treatment and control plots from November 2014 to August 2015.

Aboveground biomass

Biomass data were natural log transformed to meet assumptions of equal variances. A two-way ANOVA showed no significant effect of snow removal ($F = 0.841$, $df = 1$, $p = 0.386$) and drought ($F = 3.05$, $df = 1$, $p = 0.119$) on aboveground biomass (Figure 9). No interactions were

found ($F = 3.72$, $df = 1$, $p = 0.09$). However, the drought/snow removal plots had a higher variance (0.199) compared to the control plots (0.098). Although stress combination plots had a higher overall biomass, no significant differences were found in the plots and treatment types.

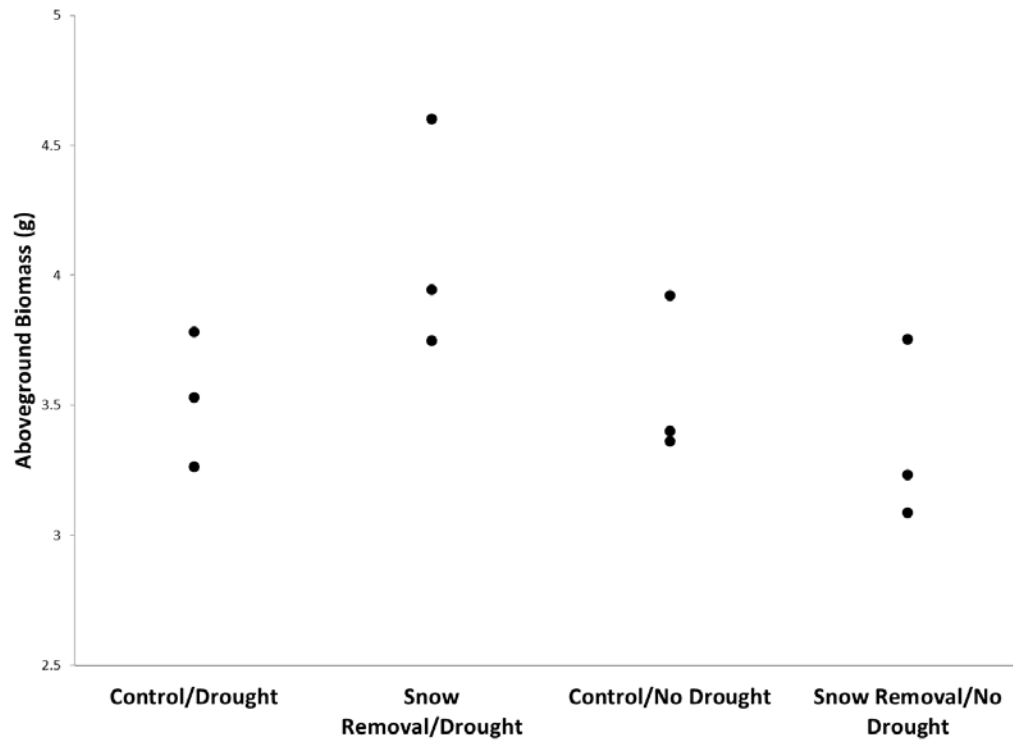


Figure 9. Aboveground biomass collected in November 2015. No difference in biomass was found between the plots and treatment types.

Soil moisture content

Soil moisture trends of the control and incomplete rain-out shelter plots were examined to determine if plots differed in available moisture (Figure 10). The experimental plots show little variance in soil water content suggesting that the plots have equal access to moisture. Soil moisture content determined from soil cores taken in November 2015 were natural log transformed. A two-way ANOVA was done to examine the effects of snow removal and drought

on soil moisture content. A significant difference in moisture was found between plots with a rain-out shelter and the control ($F=11.768$, $df= 1$, $p= 0.009$). No differences were found between the subplots of the snow removal treatment and control (Figure 11).

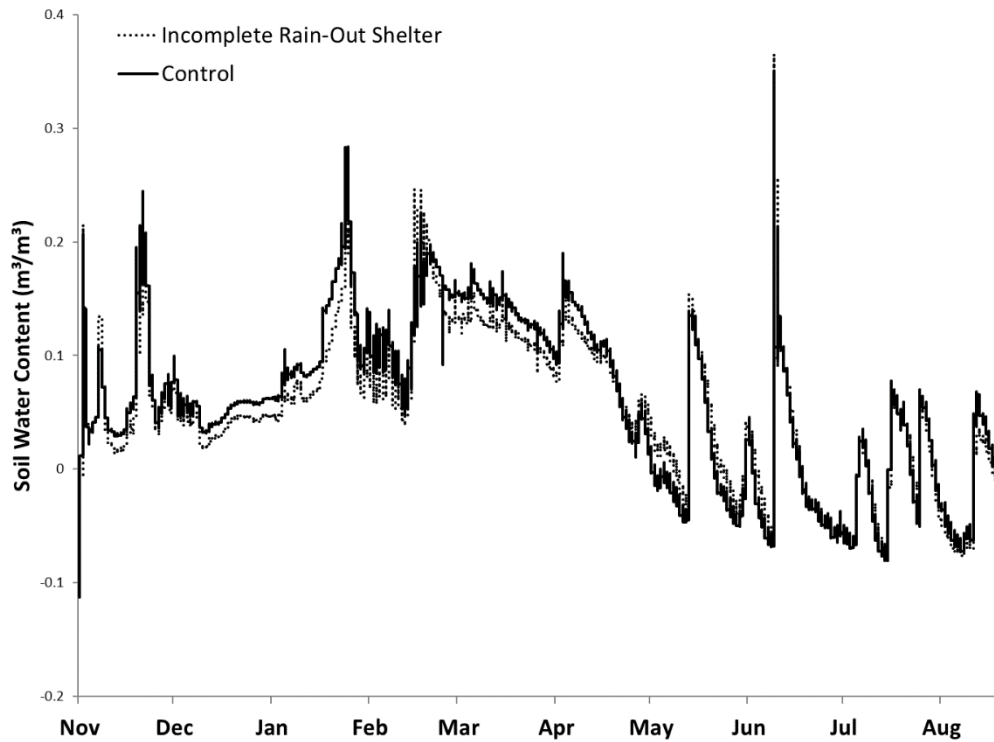


Figure 10. Pre rain-out shelter and control soil water content recorded by data loggers from November 2014 to August 2015. This demonstrates that there is little variance in moisture between plots.

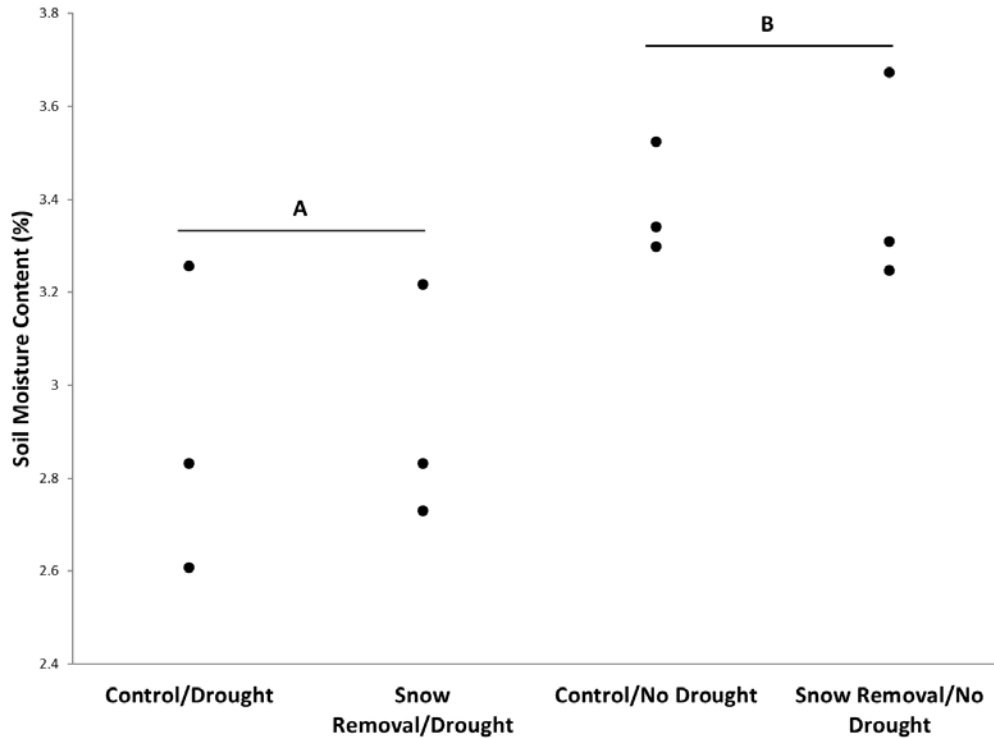


Figure 11. Soil moisture content determined from soil cores collected in November 2015. Different letters indicate significant differences between treatment types.

Discussion

This study investigated the effects of frost stress and drought in the temperate grassland, Lac du Bois Grasslands Protected Area. I hypothesized that plants exposed to the combination of frost and drought would have a higher productivity than plants exposed to either frost or drought stress alone. This hypothesis was not confirmed as aboveground biomass was not influenced by multiple or single stress events.

Removing snow increased temperature variance and resulted in negative temperature spikes. Increased exposure to colder temperatures accumulated frost stress faster, and at a higher amount. Frost stress and drought, as well as the combination of both, had no effect on

aboveground biomass. Drought was simulated by rain-out shelters which reduced soil moisture content. No moisture differences were found between the snow removal treatment and control subplots.

Snow removal

Snow was removed repeatedly over three months from the treatment subplots within the experimental plots. There was a significant difference in mean snow depth after removal in treatment plots. Over time, due to continuous snow fall, mean snow depth of treatment plots increased to similar depths of the control plots. Repeated removal of newly fallen snow was essential to ensure less snow cover was available for insulation against frost stress. However, snow removal for this study may not be frequent and consistent enough to stimulate severe frost stress to affect biomass productivity. After snow was removed on December 9th, there was a long delay before snow was removed again on January 6th, 2015. This delay may have provided the snow removal subplots with enough snow cover and insulation to reduce exposure to frost stress resulting in the aboveground biomass being unaltered. With less snow cover, temperatures were more variable and extreme in treatment plots, with a low of -8.1 °C compared to -2.9 °C in the control plots. This clearly demonstrates how snow cover provides good insulation against sub-zero temperatures. Snow removal plots experienced a higher magnitude of FSDD (105.5 °C) compared to the control (34.8 °C). This high FSDD indicates that the treatment plots were exposed to more negative temperatures which may inhibit growth and potentially damage plants. High stress degree day (SDD) and its relationship with increased damage is supported by a study done on snap beans which found that increasing SDD on water stress decreased growth and development (Helyes et al. 2006).

Aboveground biomass

Aboveground biomass was an important indicator for this study to determine if productivity was influenced by stress events. While there was a higher overall biomass for the snow removal/drought combination plots, there was no significant difference between the experimental plots. This result may be due to differences in species composition between sample sites. For example, rough fescue (*Festuca campestris*) is a bunchgrass which can form dense clumps (Anderson 2006). If, by chance, a sample site included a dense clump of rough fescue then the biomass results may be higher than a sample that did not have rough fescue. To counter this potential variance, more replication is needed.

Contrary to previous studies, aboveground biomass was not reduced when exposed to either frost stress or drought. Drought is a stressor that has one of the most negative effects on plant growth and development (Fleta-Soriano and Munné-Bosch 2016). Frost stress also has negative impacts on shoot and canopy growth as well as flowering (Briceño et al. 2014). A study done on sub-Arctic shrubs found that winter warming reduced the insulating properties of snow cover which resulted in less summer growth and an increased frequency of dead shoots (Bokhorst et al. 2009). However, my results are not consistent with these studies as collected biomass productivity showed no difference when exposed to stress.

A major drawback of my study was the timing of the fully constructed rain-out shelters which were completed at the end of August. Usually by this time, the main growing season has already ended. Aboveground biomass was collected in November to allow for some fall regrowth or stress interactions to occur. However, no interactions were found. Furthermore, it may be possible that the rain-out shelters prevented fall regrowth by reducing the required amount of

moisture to initiate growth. Thus, with little to no fall regrowth, interactions and biomass differences were not found.

Soil moisture content

Soil water content, recorded by the data loggers, was similar throughout the year among the experimental plots. This indicates that each plot received relatively the same amount of moisture with little variance, suggesting that plots were essentially equal and received the same opportunity for growth regarding moisture limitations. In November, soil cores were collected and analyzed. Soil moisture content was significantly lower in plots with rain-out shelters than the control plots and no difference was found between treatment types. A study using a comparable shelter design found results supporting my findings. Yahdjian and Sala (2002) found that soil water content was higher in control plots than plots with rain-out shelters and also found there was no significant difference between the observed and expected water interception. This is a good indicator that the rain-out shelters for this study were performing as expected reduced precipitation by at least 50%.

Conclusions

After conducting this study, I found no measureable effect on aboveground biomass of frost, drought and the combination of both. Snow removal increased frost stress by exposing plants to more variable and negative temperatures. Rain-out shelters were effective in reducing soil moisture content for drought simulation. It appears that the frost stress was not severe enough and drought stress was too late in the season to influence biomass production. These results were somewhat unexpected as previous studies looking at grassland species found that prior exposure to drought improved frost tolerance (Kreyling et al. 2012). However, we looked at frost stress on drought tolerance so it may be possible that acclimation mechanisms differ

depending on which type of stress exposure came first. This study provides baseline data for future and long term studies.

Long term studies are important for better understanding how ecosystems change over time (Magnuson 1990). The International Drought Experiment (2015) is a long term study investigating terrestrial ecosystem sensitivity to drought all around the world. This international experiment may help us understand how and why different ecosystems respond when exposed to drought (IDF 2015). Long term studies are especially important for understanding stress interactions due to lagged or delayed stress effects that are not clear until after some period of time (Walter et al. 2013). This long term study may help us better understand and predict how terrestrial ecosystems will respond in the face of climate change. It may also provide some insight on the consequences of ecological stress memory and if there is a positive or negative effect on plant communities.

We plan to continue this experiment over the next five years. The rain-out shelters have been completed and will be able to reduce precipitation throughout the entire growing season to simulate drought. I predict that impacts on aboveground biomass will become more apparent over the next few years as stress becomes more severe. This long term study may provide better insights to plant responses during changing climate and be able to provide suggestions for adapting to climate change.

LITERATURE CITED

- Anderson GD. 2006. *Festuca campestris* Rydberg (rough fescue): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available at: <http://www.fs.fed.us/r2/projects/scp/assessments/festucacampestris.pdf> Accessed April 15, 2016.
- Backhaus S, Kreyling J, Grant K, Beierkuhnlein C, Walter J, Jentsch A. 2014. Recurrent mild drought events increase resistance toward extreme drought stress. *Ecosystems* 17:1068–1081.
- Batalla I, Knudsen MT, Mogensen L, Hierro Ó del, Pinto M, Hermansen JE. 2015. Carbon footprint of milk from sheep farming systems in Northern Spain including soil carbon sequestration in grasslands. *Journal of Cleaner Production* 104:121–129.
- Beck EH, Fettig S, Knake C, Hartig K, Bhattarai T. 2007. Specific and unspecific responses of plants to cold and drought stress. *Journal of biosciences* 32:501–510.
- Bohnert HJ. 2000. What makes desiccation tolerable. *Genome Biol* 1(2):1-4
- Bokhorst SF, Bjerke JW, Tømmervik H, Callaghan TV, Phoenix GK. 2009. Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. *Journal of Ecology* 97:1408–1415.
- Boval M, Dixon RM. 2012. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. *Animal* 6:748–762.
- Briceño VF, Harris-Pascal D, Nicotra AB, Williams E, Ball MC. 2014. Variation in snow cover drives differences in frost resistance in seedlings of the alpine herb *Aciphylla glacialis*. *Environmental and Experimental Botany* 106:174–181.
- Bruce TJA, Matthes MC, Napier JA, Pickett JA. 2007. Stressful “memories” of plants: Evidence and possible mechanisms. *Plant Science* 173:603–608.
- Chapin FS, Matson PA, Mooney HA. 2002. *Principles of terrestrial ecosystem ecology*. New York: Springer

- Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Sarvalho I, Faria T, Pinheiro C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany* 89: 907-916.
- Choler P. 2015. Growth response of temperate mountain grasslands to inter-annual variations in snow cover duration. *Biogeosciences* 12:3885–3897.
- Committee on the Status of Endangered Species in Canada (COSEWIC). 2001. Canadian Species at Risk. Canadian Wildlife Service, Environment Canada. Ottawa
- Conrath U, Beckers GJM, Flors V, Garcia-Agustin P, Jakab G, Mauch F, Newman MA, Pieterse CMJ, Poinssot B, Pozo MJ, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-Mani B. 2006. Priming: getting ready for battle. *Mol Plant Microbe Interact* 19:1062-1071
- Ćuk K, Gogalo M, Tkalec M, Vidaković-Cifrek Ž. 2010. Transgenerational stress memory in *Arabidopsis thaliana* (L.) Heynh.: antioxidative enzymes and HSP70. *Acta Botanica Croatica* 69:183–197.
- Fleta-Soriano E, Munné-Bosch S. 2016. Stress memory and the inevitable effects of drought: A physiological perspective. *Frontiers in Plant Science* 7.
- Fraser LH, Greenall A, Carlyle CN, Turkington R, Ross Friedman C. 2009. Adaptive phenotypic plasticity of *Pseudoroegneria spicata*: response of stomatal density, leaf area and biomass to changes in water supply and increased temperature. *Annals of Botany* 103: 769-775.
- Fraser LH and Carlyle CN 2011. Is size of spotted knapweed (*Centaurea stoebe* L.) patch related to the effect on soil and vegetation properties? *Plant Ecology* 212: 975-983.
- Frost CJ, Mescher MC, Carlson JE, De Moraes CM. 2008. Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology* 146:818–824.
- Google Earth. 2016. Google Earth. URL <http://earth.google.com>
- Helyes L, Pék Z, McMichael B. 2006. Relationship between the stress degree day index and biomass production and the effect and timing of irrigation in snap bean (*Phaseolus vulgaris* var. *nanus*) stands: results of a long-term experiments. *Acta Botanica Hungarica* 48:311–321.

Holzmüller, E.J., and José, S. 2013. What makes alien plants so successful? Exploration of the ecological basis. In *Invasive plant ecology*. Edited by S. José, H.P. Singh, D.R. Batish, and R.K. Kohli. CRC Press.

International Drought Experiment (IDF). 2015. Drought-Net A global network to assess terrestrial ecosystem sensitivity to drought. Available at: <http://wp.natsci.colostate.edu/droughtnet/> Accessed March 24, 2016.

IPCC. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability*. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds., Cambridge University Press, Cambridge, UK, 976pp.

IPCC. 2012. *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation*. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change [Field CB, V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK, and New York, NY, USA, 582 pp.

Jérôme E, Beckers Y, Bodson B, Heinesch B, Moureaux C, Aubinet M. 2014. Impact of grazing on carbon dioxide exchanges in an intensively managed Belgian grassland. *Agriculture, Ecosystems & Environment* 194:7–16.

Kinoshita T, Seki M. 2014. Epigenetic memory for stress response and adaptation in plants. *Plant and Cell Physiology* 55:1859–1863.

Kleunen, M., Dawson, W., & Maurel, N. 2015. Characteristics of successful alien plants. *Molecular Ecology*, 24(9), 1954-1968.

Kodra E, Steinhäuser K, Ganguly AR. 2011. Persisting cold extremes under 21st-century warming scenarios: Global warming and cold extremes. *Geophysical Research Letters* 38.

Kreyling J, Thiel D, Simmnacher K, Willner E, Jentsch A, Beierkuhnlein C. 2012. Geographic origin and past climatic experience influence the response to late spring frost in four common grass species in central Europe. *Ecography* 35:268–275.

Lee RN, Bradfield GE, Krzic M, Newman RF, Cumming WFP. 2014. Plant community – soil relationships in a topographically diverse grassland in southern interior British Columbia, Canada. *Botany* 92:837–845.

- Magnuson JJ. 1990. Long-term ecological research and the invisible present. *BioScience* 40:495-501.
- McCulloch, JA. 2013. Effects of changing precipitation patterns and clipping on the shrub-steppe grassland plant communities of the southern interior of British Columbia. Thompson Rivers University.
- Medeiros JS, Pockman WT. 2011. Drought increases freezing tolerance of both leaves and xylem of *Larrea tridentata*: Drought increases freezing tolerance in *Larrea tridentata*. *Plant, Cell & Environment* 34:43–51.
- Ministry of Environment, Lands and Parks. 2000. Lac du Bois Grasslands Park Management Plan Background Document. BC Parks, Thompson River District, Kamloops BC.
- Ministry of Environment, Lands and Parks. 2004. Lac du Bois Grasslands Provincial Park Management Plan. BC Parks, Thompson River District, Kamloops, BC
- Nalley D, Adamowski J, Khalil B, Ozga-Zielinski B. 2013. Trend detection in surface air temperature in Ontario and Quebec, Canada during 1967–2006 using the discrete wavelet transform. *Atmospheric Research* 132-133:375–398.
- Naudts K, Van den Berge J, Janssens IA, Nijs I, Ceulemans R. 2013. Combined effects of warming and elevated CO₂ on the impact of drought in grassland species. *Plant and Soil* 369:497–507.
- Nutrient Network. 2008. Experimental protocol.
- Pastor V, Luna E, Mauch-Mani B, Ton J, Flors V. 2013. Primed plants do not forget. *Environ Exp Bot* 94:46-56.
- Polle, A., Kroniger, W., Rennenberg, H., 1996. Seasonal fluctuations of ascorbate-related enzymes: acute and delayed effects of late frost in spring on antioxidative systems in needles of Norway spruce (*Picea abies* L.). *Plant and Cell Physiology* 37, 717–725.
- Reichmann LG, Sala OE. 2014. Differential sensitivities of grassland structural components to changes in precipitation mediate productivity response in a desert ecosystem. Whitehead D, editor. *Functional Ecology* 28:1292–1298.

- Roychoudhury A, Banerjee A, Lahiri V. 2015. Metabolic and molecular-genetic regulation of proline signaling and its cross-talk with major effectors mediates abiotic stress tolerance in plants. *Turkish Journal of Botany* 39:887–910.
- Ryswyk A van, McLean A, Marchand LS. 1966. The climate, native vegetation, and soils of some grasslands at different elevations in British Columbia. *Canadian Journal of Plant Science* 46:35–50.
- Sanaullah M, Rumpel C, Charrier X, Chabbi A. 2012. How does drought stress influence the decomposition of plant litter with contrasting quality in a grassland ecosystem? *Plant and Soil* 352:277–288.
- Smith MD. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research: Defining extreme climate events. *Journal of Ecology* 99:656–663.
- Spittlehouse, DL. 2008. *Climate Change, Impacts, and Adaptation Scenarios: Climate Change and Forest and Range Management in British Columbia*. Victoria, BC.
- Sun S, Sun G, Caldwell P, McNulty S, Cohen E, Xiao J, Zhang Y. 2015. Drought impacts on ecosystem functions of the U.S. National Forests and Grasslands: Part II assessment results and management implications. *Forest Ecology and Management* 353:269–279.
- Tahkokorpi M, Taulavuori K, Laine K, Taulavuori E. 2007. After-effects of drought-related winter stress in previous and current year stems of *Vaccinium myrtillus* L. *Environmental and Experimental Botany* 61:85–93.
- Ton J, Jakab G, Tonquin V, Flors V, Lavicoli A, Maeder MN, Metraux JP, Mauch-Mani B. 2005. Dissecting the aminobutyric acid-induced priming phenomenon in *Arabidopsis*. *The Plant Cell Online* 17:987–999.
- Walter J, Nagy L, Hein R, Rascher U, Beierkuhnlein C, Willner E, Jentsch A. 2011. Do plants remember drought? Hints towards a drought-memory in grasses. *Environmental and Experimental Botany* 71:34–40.
- Walter J, Jentsch A, Beierkuhnlein C, Kreyling J. 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany* 94:3–8.

Wikeem, B. Wikeem, S. 2004. Grasslands of British Columbia.

Yahdjian L, Sala OE. 2002. A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133:95–101.

Yamaguchi-Shinozaki K, Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57:781–803.

Yusa A, Berry P, J.Cheng J, Ogden N, Bonsal B, Stewart R, Waldick R. 2015. Climate Change, Drought and Human Health in Canada. *International Journal of Environmental Research and Public Health* 12:8359–8412.